

# Editing the microbiome

After years of monitoring the body's microbial communities, researchers are now starting to modify them to treat disease.

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Put the gut microbes from an obese mouse into the body of a lean one, and the latter will pack on weight—even without changes to its diet, activity, or other habits (1). Such studies have made it increasingly clear that an organism's commensal microbes can have powerful health effects, including in humans. A decade of research suggests that our microbiota help drive brain development, spur immune-system development or allergies (2), and might trigger certain cancers. These observations have spawned industries that offer probiotics, prebiotics, personal microbiota sequencing, and more.

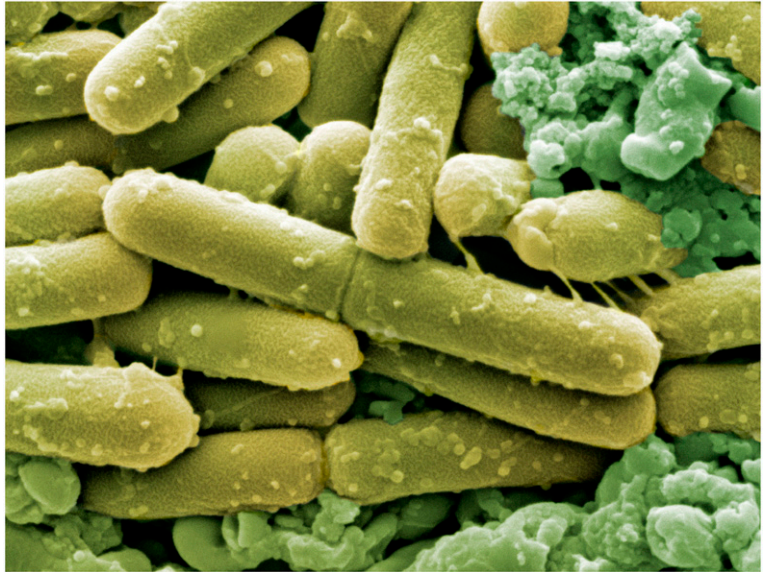
But few therapeutics actually alter—much less improve—our commensal microflora, in part because watching these microbial dynamics play out is still a step short of understanding their mechanisms well enough to intervene effectively and safely.

Already, fecal microbial transplants (FMTs) have demonstrated both the promise and peril of manipulating microbiota. For desperate patients fighting persistent intestinal *Clostridium difficile* infections, the transplants, in which the entirety of bacteria from a healthy person's intestines are transferred to those of an ill individual, can offer relief (3). Yet even when FMT works, there's no way to standardize or quantify its effects. Recent cases of severe side effects—including one death—led the US Food and Drug Administration to halt all clinical trials of FMT until added safety measures are put in place (4).

"We really don't know the long-term consequences of an FMT," says microbiologist Casey Theriot of North Carolina State University in Raleigh. "It saves lives, but we don't want to use it for everything. We need more defined bacterial therapeutics."

Theriot and others are working to create targeted therapies and tools for precision editing of microbiomes to treat disease. Based on understanding how inner ecosystems function, change, and govern our health in specific situations, researchers can remove pathogens or add metabolic functions without disrupting the larger community.

"There's been a big change in the last few years where we've shifted from simply seeing what microbes



Infections of *Clostridium difficile* (pictured in scanning electron micrograph) are a particularly attractive target for microbiome researchers, who generally have a good understanding of the pathogen's interaction with commensal microbes and the gut environment. Image credit: Science Source/Paul Gunning.

there are in health and disease to connecting them to host biology," says microbiologist Justin Sonnenburg of Stanford University in Palo Alto, CA. "It's been a transition to a functional understanding, and is leading to an era of rationally designing microbiota."

## Targeting Single Species

A bane of patients hospitalized for other illnesses, *C. difficile* infections are a particularly attractive target for researchers because the pathogen's interaction with commensal microbes and the gut environment is well understood. "We know exactly what to go after in these infections," Theriot says. "There's a very clear relationship between the microbiota, its metabolites, and *C. difficile* infections."

In a healthy gut, commensal Clostridia and other species block *C. difficile* from proliferating, in part by competing for the same nutrients that the pathogen



To deter bad bacteria, microbiologist Andreas Bäumlér, of University of California, Davis, makes the gut environment less friendly to carbapenem-resistant Enterobacter species such as *Klebsiella pneumoniae* (pictured in scanning electron micrograph, interacting with a human neutrophil), which can endanger vulnerable hospitalized patients. Image credit: Flickr/NIAID, licensed under [CC BY 2.0](#).

needs. But antibiotics obliterate these commensals, and in the absence of these beneficial microbes and the chemical signals they produce, *C. difficile* can establish severe infections (5). Patients experience diarrhea, nausea, and abdominal pain—and are again prescribed an antibiotic to treat their symptoms. The drugs can cure infections in most patients with *C. difficile*, but at least 30% of these patients spiral into worsening infections because the pathogenic Clostridia return more quickly than the commensals, necessitating yet another round of antibiotics (6). This cycle of reinfection can last for years—and it is often why desperate patients resort to drastic measures such as DIY fecal transplants.

Theriot and her team wanted to target *C. difficile* by using methods that would not inhibit commensal bacteria, so the researchers turned to an engineered bacteriophage produced by Morrisville, NC-based startup Locus Biosciences. Phages target specific bacterial species, and previous studies have suggested that phage use might be an effective strategy to treat recurrent *C. difficile* infections (7). The company engineered phages that naturally target *C. difficile* to carry DNA sequences in their own genomes that encode a gene-editing system known as CRISPR-Cas3. The Cas3 enzyme “chews DNA up like a Pac-Man,” dicing up the entire targeted bacterium’s

genome, says Theriot, who is a scientific advisor for the company.

In unpublished work, Theriot’s team found that in mice experimentally infected with *C. difficile*, a twice-daily dose of these Cas3-bearing phages over three days reduced clinical signs of disease such as edema, weight loss, and damage to the gut epithelial surface, and that levels of pathogens were significantly reduced. Theriot says the work is an initial proof of concept that the strategy works.

Such a strategy amounts to a direct assault. Another approach tries instead to make the microenvironment of the target pathogen inhospitable. Microbiologist Andreas Bäumlér at the University of California, Davis, targets the metabolic disruptions to human cells caused by antibiotics. The idea is to make the environment less friendly to carbapenem-resistant Enterobacter (CRE) species such as *Klebsiella pneumoniae* and *Escherichia coli*, which are also major threats to vulnerable hospitalized patients.

Drugs that destroy the gut microflora silence one half of the chemical conversation between microbial and human cells. Under these circumstances, intestinal epithelial cells start to produce higher levels of oxygen and nitrates—creating an environment in which CRE infections can take root (8). Because patients may still be taking antibiotics, restoring a full, healthy microbiome using probiotic bacteria isn’t feasible, because these strains are also sensitive to antibiotics.

So Bäumlér and his colleagues have tried restoring key elements of a healthy environment by reintroducing missing microbial cues that instruct intestinal cells’ activities. Their team used a “faux-biotic”—a common drug called 5-amino salicylic acid (5-ASA) that’s already used to treat inflammatory bowel disease in combination with a probiotic strain of *E. coli*. They found that the cocktail protected model animals from colonization by CRE. The treatment “worked in the same way as a full FMT,” Erin Olsan, a former postdoc in Bäumlér’s lab, told an audience last June at the American Society for Microbiology annual meeting in San Francisco, CA (9).

Bäumlér likens these infections—and the challenges of treating them—to a game of musical chairs. When our microbiomes are being established, our diet, immune system, and a host of other factors determine what meal options microbes have within the large intestine, thereby deciding which commensals will dine well and proliferate in our guts. In a healthy, mature gut ecosystem, each place at the dinner table is filled, leaving no room for pathogens. This so-called colonization resistance makes the healthy microbiota resistant to change—and also makes it tricky to create therapeutics that have long-lasting effects.

In diseased or antibiotic-dosed microbiota, many diners have been knocked off their usual seats. Using faux-biotics or small molecules produced by the gut microflora is akin to placing a jacket over a chair to claim the spot, Bäumlér says.

Although these approaches are proving effective against clear targets such as *C. difficile* or CREs, they

may prove trickier to implement in other diseases. “The problem often comes in because in diseases such as inflammatory bowel disease, obesity, or colon cancer, we don’t know what microbial targets are important,” Theriot says.

### Microbial “Gene Therapy”

Pinning down a single culprit species may be near impossible. But it also may not be necessary. “The one-bug-at-a-time approach is good for understanding bacterial physiology,” says Harris Wang of Columbia University in New York. “But in the context of complex communities, you might never be able to kind of decompose it down into the individual elements that way.” For the field to advance, researchers must study and experimentally manipulate the community as a whole, not just individual lab-grown strains of bacteria, he says.

To understand whether a certain gene or metabolic function helps a microbe colonize the gut, the surest confirmation is to remove that gene or metabolic pathway and test the bacteria’s ability to colonize, he explains. The need for tools to manipulate microbiomes in these precise ways—both to add and to remove functions—is what drives Wang’s research, he says.

Wang’s group aims to perform what he calls “a microbial gene therapy of sorts,” delivering selected genes and pathways to an established, native microbial community, which can then harbor those abilities and transmit them stably over time.

To that end, his team has focused on mobile pieces of DNA known as plasmids, which bacteria can copy and hand out to neighboring cells without changing their own or the recipient’s chromosomes. Unlike phages, which have a very specific set of hosts, plasmids can be tweaked to be passed around either en masse or to specific recipients. In a recent study, the team engineered *E. coli* to carry a broad host-range plasmid that could be transferred between bacteria that touched one another. Using a gene to produce a fluorescent marker, they found that gut bacteria in mice treated with this *E. coli* strain carried the marker in a wide range of commensal species (10). In principle, the technique could be used to add genes that encode functions such as metabolizing a toxin or a specific nutrient. Even if the engineered *E. coli* fail to establish themselves in the gut because they can’t find a spot at the dinner table, the plasmids they’ve shared should persist in native species.

Another tactic to add functions—and make sure that they persist—is to add a special dish to the dinner menu for the engineered microbes. Last year, Sonnenburg’s team published a study demonstrating how *Bacteroides* species—the most abundant bacteria in the typical American adult’s gut—could be modulated to digest dietary oxalates. These chemicals are commonly found in tomatoes, leafy greens, dark chocolate, and a host of other foods. Although harmless to most, oxalate-rich foods can cause kidney stones to reform in patients who’ve previously had them. Sonnenburg and his colleagues engineered *Bacteroides*

to carry genes encoding oxalate-degrading enzymes. They also added genes to digest porphyrin, a sugar from edible seaweeds that’s uncommon in the standard Western diet and acts as a control molecule that’s required for the engineered microbes to survive. When mice were fed this polysaccharide and then treated with the engineered bacteria, the bacteria had a special niche to themselves in the gut microbial community (11).

“It allows the bacteria to work independent of the background microbiota to reach high, reproducible levels of colonization,” Sonnenburg says. “People generally have a tough time controlling their diet. So the idea here is that if you had this therapeutic microbe in your gut, you wouldn’t have to pay as much attention to avoiding oxalates in your diet.”

In a similar vein, engineering microbes native to a particular host’s microbiome may help to ensure their survival in a niche they already inhabit successfully. Amir Zarrinpar of University of California, San Diego works with microbes commonly found in the native microflora, in part because of an accidental discovery. His team had been working with *E. coli* Nissle, a common lab strain that others are exploring for therapeutic uses, but found that their plasmid-carrying bacteria failed to proliferate well within animals. While doing that experiment, a former postdoc in Zarrinpar’s lab, Steven Brown, found that another strain of *E. coli*—one native to the mouse gut—

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**—Amir Zarrinpar**

seemed to fare better. They switched gears to express a plasmid carrying a fluorescent marker in the resident microbe instead, and the engineered native strain was significantly better at colonizing mouse intestines (12). If this strain were modified to carry genes encoding specific metabolic functions, it could theoretically re-integrate and persist in the gut community without the need for additional nutrients such as porphyrin.

“Engineering native bacteria isn’t as straightforward because they have innate defense mechanisms to prevent their DNA from being manipulated,” Zarrinpar says. “But if we study these features well, we can also use them to protect the DNA we insert into these bacteria.”

Another advantage with native bacteria, he says, is that they can be used to target specific locations in the body: bacteria that colonize the mucus layer of the upper colon are distinct from those that live deep within intestinal folds known as crypts. So simply choosing the right strain could deliver a function to one location or the other. “It’s worth better studying native bacteria because they can be powerful vectors,” Zarrinpar says.

For now, these studies are mostly seeking proof of principle using genes like *GFP* to produce a fluorescent marker that shows whether the method works.

“The next big challenge is to demonstrate that we can functionally manipulate the microbiome,” Zarrinpar adds. “And secondly, to show we can control it in a way that can be translated to humans.”

### Interventions and Insights

Eventually, these approaches to editing microbiomes could be used not only to develop therapeutics, but also to build sensors to track microbial metabolism. And as research tools, they will continue to broaden researchers’ understanding of the mechanisms microbes use to influence human health and disease.

Still, a handful of start-ups launched from academic labs are already taking some microbiome editing techniques into the clinic, with several in phase I clinical trials. Locus Biosciences’ Cas3-bearing phages are being tested for *C. difficile*, urinary tract *E. coli* infections, and others. Cambridge, MA-based Synlogic has tested several strains of a commensal *E. coli* tweaked to carry genes that target deficient metabolic pathways, such as those in phenylketonuria, a genetic disease in which patients lack enzymes to degrade certain amino acids. Their SYN1618 strain delivers enzymes that degrade phenyl-alanine, one of these amino acids, in the small intestine (13). San Francisco, CA-based Siolta Therapeutics is testing a defined mix

of bacteria that can reduce allergic responses such as asthma in animal models (14).

Challenges and unanswered questions certainly remain. Therapeutic strategies will need to overcome the native microflora’s resistance to new residents, whether temporary or permanent. Those trying to engineer native bacteria need to identify genetic tools and growth conditions, because many of these species will never have been cultured or modified in laboratory experiments. When inserting pieces of DNA such as plasmids, researchers will need to learn how to control the spread of these mobile elements in the wild. And perhaps most importantly, results don’t always translate from mice to humans—or even from early human studies to later ones.

Ideally, Zarrinpar says, researchers will use these tools to move beyond using microbes at all. After years of watching the workings of our microbiota, and now engaging in rational bug design, researchers will be able to identify small molecules or therapeutics that mimic or override microbial functions. “The perfect microbiome treatments will not use the microbiome,” he says. “Instead, we’ll identify the levers that the microbiome pushes to elicit physiological changes, and then act on those levers ourselves instead of relying on bacteria.”

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